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Application of: Nielsen *et al.*

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For: RECOMBINANT TISSUE PROTECTIVE CYTOKINES AND ENCODING NUCLEIC ACIDS THEREOF FOR THE PROTECTION, RESTORATION AND ENHANCEMENT OF RESPONSIVE CELLS, TISSUES AND ORGANS

Attorney Docket No: 10165-022-999

AMENDMENT UNDER 37 C.F.R. § 1.111

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the non-final Office Action dated December 31, 2007 ("Office Action") and pursuant to the Rules of Practice, please enter the following amendments and consider the following remarks. Enclosed herewith are: (i) a Petition for Extension of Time for three (3) months from March 31, 2008 to and including June 30, 2008 with a provision authorizing payment of the required fee (in duplicate); (ii) an Amendment Fee Transmittal Sheet (in duplicate); (iii) Transmittal of Substitute Sequence Listing (in duplicate); (iv) Substitute Sequence Listing in paper and computer-readable format; (v) Exhibit A: filing receipt for Application Serial No. 10/188,905; (vi) Exhibit B: copies of Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, pp. 994 and 1005, entries for "rejuvenate" and "restore," respectively; (vii) Appendix A: Replacement table at pages 69-71 of specification; (viii) a Declaration of Michael L. Brines, M.D., Ph.D. ("Brines Declaration"), with Appendices A-E; and (ix) Supplemental Information Disclosure Statement (in duplicate) with revised form PTO 1449 entitled "List of References Cited By Applicant," accompanied by copies of references A73-A81, B09-B43, and C123-C239.

Applicants respectfully request that the amendments and remarks made herein be entered into the record of the instant application. Please charge all required fees to Deposit Account No. 50-3013.

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph at p. 9, l. 27 to p. 10, l. 5 with the following amended paragraph:

Moreover, such aforementioned recombinant tissue protective cytokines may be further modified by having a chemical modification of one or more amino acids, such as described in the following co-pending applications: PCT application serial no. PCT/US01/49479, filed December 28, 2001, U.S. Patent Application Serial No. 09/753,132 filed December 29, 2000, and U.S. Patent Application ~~Attorney Docket No. KW00-009C02-US~~ 10/188,905 filed July 3, 2002, each of these applications is incorporated herein by reference in their entirety. These further chemical modifications may be used to enhance the tissue protective activities of the recombinant tissue protective cytokines or suppress any effects the recombinant tissue protective cytokines may have on bone marrow. In a further embodiment, the additional chemical modification is provided to restore solubility of the molecule that may be reduced as a result of the aforementioned genetic modification, such as chemically adding a positive or negative charge to the molecule if a charged amino acid residue is changed to an uncharged residue.

Please replace the paragraph at p. 10, ll. 7-16, with the following amended paragraph:

By way of non-limiting examples, recombinant tissue protective cytokines of the invention include human erythropoietin mutein S100E ~~(SEQ ID NO:5)~~ (SEQ ID NO:62), human erythropoietin mutein K45D ~~(SEQ ID NO:6)~~ (SEQ ID NO:44), and any of the nonerythropoietic yet cellular protective recombinant tissue protective cytokines or those able to benefit a responsive cell, tissue or organ, that are described in Elliott *et al.*, 1997, Blood 89:493-502; Boissel *et al.*, Journal of Biological Chemistry, vol. 268, No. 21, pp. 15983-15993 (1993); Wen *et al.*, Journal of Biological Chemistry, vol. 269, No. 36, pp. 22839-22846 (1994); and Syed *et al.*, Nature, vol. 395, pp. 511-516 (1998), which are incorporated herein by reference in their entireties. The present invention is directed to methods for the use of any of the aforementioned recombinant tissue protective cytokines for the protection, restoration, and enhancement of responsive cells, tissues, and organs.

Please replace the paragraph at p. 16, ll. 9-16, with the following amended paragraph.

According to one aspect of the invention, there is provided an isolated nucleic acid molecule that comprises a nucleotide sequence which encodes a polypeptide comprising the recombinant tissue protective cytokine as described herein above. In one embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence of nucleotide residues 5461 through 6041 of the ~~vecotor contruct~~ vector construct of SEQ ID NO: 208, nucleotide residues 5461 through 6041 of SEQ ID NO: 209, nucleotide residues 5461 through 6041 of SEQ ID NO: 210, nucleotide residues 5461 through 6041 of SEQ ID NO: 211, or nucleotide residues 5461 through 6041 of ~~SEQ ID NO: 212~~ SEQ ID NO:5.

Please replace the paragraph at p. 20, ll. 3-17 with the following amended paragraph:

The invention further provides for the use of a recombinant tissue protective cytokine as described herein above, that lacks at least one erythropoietic activity selected from the group consisting of increasing hematocrit, vasoactive action (vasoconstriction/ vasodilatation), hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes, for the preparation of a pharmaceutical composition for the protection against and prevention of a tissue injury as well as the restoration of and rejuvenation of tissue and tissue function in a mammal. In one embodiment, the injury is caused by a seizure disorder, multiple sclerosis, stroke, hypotension, cardiac arrest, ischemia, myocardial infarction, inflammation, age-related loss of cognitive function, radiation damage, cerebral palsy, neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Leigh's disease, AIDS dementia, memory loss, amyotrophic lateral sclerosis, alcoholism, mood disorder, anxiety disorder, attention deficit disorder, hyperactivity, autism, ~~Creutzfeld-Jakob~~ Creutzfeldt-Jakob disease, brain or spinal cord trauma or ischemia, heart-lung bypass, chronic heart failure, macular degeneration, diabetic neuropathy, diabetic retinopathy, glaucoma, retinal ischemia, or retinal trauma.

Please replace the paragraph at p. 39, l. 8 to p. 40, l. 14 with the following amended paragraph:

Recombinant tissue protective cytokines of the invention include erythropoietin muteins, that maintain partial or full erythropoietic activity. Erythropoietin is a glycoprotein

hormone which in humans has a molecular weight of about 34 kDa. The mature protein comprises 165 amino acids (SEQ ID NO:10), and the glycosyl residues comprise about 40% of the weight of the molecule. The forms of recombinant tissue protective cytokine useful in the practice of the present invention encompass at least a single amino acid change in naturally-occurring, synthetic and recombinant forms of the following human and other mammalian erythropoietin-related molecules: erythropoietin, asialoerythropoietin, deglycosylated erythropoietin, erythropoietin analogs, erythropoietin mimetics, erythropoietin fragments, hybrid erythropoietin molecules, erythropoietin receptor-binding molecules, erythropoietin agonists, renal erythropoietin, brain erythropoietin, oligomers and multimers thereof, and congeners thereof. Such equivalent recombinant tissue protective cytokines include mutant erythropoietins, which may contain substitutions, deletions, including internal deletions, additions, including additions yielding fusion proteins, or conservative substitutions of amino acid residues within and/or adjacent to the amino acid sequence, but that result in a "silent" change, in that the change produces a functionally equivalent erythropoietin mutein or recombinant tissue protective cytokine. In a preferred embodiment, the recombinant tissue protective cytokine is nonerythropoietic, i.e. lacking or exhibiting diminished erythropoietic activity. Conservative amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Alternatively, non-conservative amino acid changes, and larger insertions and deletions may be used to create functionally altered recombinant tissue protective cytokines. Such mutants can be used to alter erythropoietin properties in desirable ways. For example, in one embodiment, an erythropoietin useful for the practice of the invention can be a recombinant tissue protective cytokine altered in one or more amino acids within the four functional domains of erythropoietin which affect receptor binding: VLQRY (SEQ ID NO:1) and/or TKVNFYAW (SEQ ID NO:2) and/or SGLRSLTTL (SEQ ID NO:3) and/or SNFLRG (SEQ ID NO:4). In another embodiment, erythropoietins containing mutations in the surrounding areas of the molecule which affect the kinetics or

receptor-binding properties of the molecule can be used. Determining which alterations, or which positions in the domains will effect binding can be accomplished using standard methods. For example, the domains may be altered by pair-wise alanine mutations (ala-scanning mutagenesis) followed by measurement of binding kinetics of mutants to examine the effect on binding to a receptor (Bernat *et al.*, 2003, PNAS 100:952-957; Wells *et al.*, 1989, Science 244:1081-1085).

Please replace the paragraph at p. 42, ll. 1-23 with the following amended paragraph:

Furthermore, derivative recombinant tissue protective cytokine molecules desirable for the uses described herein may be generated by guanidination, amidination, carbamylation (carbamoylation), trinitrophenylation, acylation such as acetylation or succinylation, nitration, or modification of arginine, aspartic acid, glutamic acid, lysine, tyrosine, tryptophan, or cysteine residues or carboxyl groups, among other procedures, such as limited proteolysis, removal of amino groups, and/or mutational substitution of arginine, lysine, tyrosine, tryptophan, or cysteine residues by molecular biological techniques to produce erythropoietin muteins or recombinant tissue protective cytokines which maintain an adequate level of activities for specific organs and tissues but not for others, such as erythrocytes (*e.g.*, Satake *et al*; 1990, *Biochim. Biophys. Acta* 1038:125-9; incorporated herein by reference in its entirety[[)]], in which *in vivo* biological activity was determined by the polycythemic mouse bioassay). One non-limiting example as described hereinbelow is the modification of erythropoietin arginine residues by reaction with a glyoxal such as phenylglyoxal (according to the protocol of Takahashi, 1977, *J. Biochem.* 81:395-402). As will be seen below, such a recombinant tissue protective cytokine molecule fully retains the neurotrophic effect of erythropoietin. Such recombinant tissue protective cytokine molecules are fully embraced for the various uses and compositions described herein. In addition, these chemical modifications may be further used to enhance the protective effects of the recombinant tissue protective cytokines or neutralize any changes in the charge of the molecule resulting from the amino acid mutation of the native erythropoietin. Such modifications are described in co-pending applications:[[,]] serial no. PCT/US01/49479, filed December 28, 2001; serial no. 09/753,132, filed December 29, 2000 and ~~Attorney's Docket~~

~~No. KW00-009C02-US~~ serial no. 10/188,905, filed July 3, 2002, all of which are incorporated herein in their entireties.

Please replace the paragraph at p. 47, ll. 11-22 with the following amended paragraph:

Further to the above-mentioned erythropoietin modifications useful herein, the following discussion expands on the various recombinant tissue protective cytokines of the invention. As described in Elliott *et al.*, Boissel *et al.*, and Wen *et al.*, mentioned above, the following erythropoietin muteins are useful for the purposes described herein, and may be provided in a pharmaceutical composition for the methods herein. In the mutein nomenclature used throughout herein, the changed amino acid is depicted with the native amino acid's one-letter code first, followed by its position in the erythropoietin molecule, followed by the replacement amino acid one-letter code. For example, "human erythropoietin S100E" or "recombinant ~~tisue protectiv~~ tissue protective cytokine S100E" refers to a human erythropoietin molecule in which, at amino acid position 100 of the mature erythropoietin, a serine has been changed to glutamic acid. Such muteins useful for the practice of the present invention include but are not limited to human erythropoietin with at least one of the following amino acid changes:

Please replace the paragraph at p. 68, ll. 6-25 with the following amended paragraph:

Various neuropsychologic disorders which are believed to originate from excitable tissue damage are treatable by the instant methods. Chronic disorders in which neuronal damage is involved and for which treatment by the present invention is provided include disorders relating to the central nervous system and/or peripheral nervous system including age-related loss of cognitive function and senile dementia, chronic seizure disorders, Alzheimer's disease, Parkinson's disease, dementia, memory loss, amyotrophic lateral sclerosis, multiple sclerosis, tuberous sclerosis, Wilson's Disease cerebral and progressive *supra*nuclear palsy, Guam disease, Lewy body dementia, prion diseases, such as spongiform encephalopathies, *e.g.*. Creutzfeldt-Jakob disease, Huntington's disease, myotonic dystrophy, ~~Freidrich's~~ Friedrich's ataxia and other ataxias, as well as Gilles de la Tourette's syndrome, seizure disorders such as epilepsy and chronic seizure disorder, stroke, brain or spinal cord trauma, AIDS dementia, alcoholism, autism, retinal ischemia, glaucoma, autonomic function

disorders such as hypertension and sleep disorders, and neuropsychiatric disorders that include, but are not limited to, schizophrenia, schizoaffective disorder, attention deficit disorder hyperactivity, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, panic disorder, as well as unipolar and bipolar affective disorders. Additional neuropsychiatric and neurodegenerative disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM), the most current version, IV, of which in incorporated herein by reference in its entirety.

Please replace the paragraph at p. 89, ll. 14-20 with the following amended paragraph:

The PCR product was cloned between the Xho I and Xba I sites in pCiNeo mammalian expression vector (Promega). The clones were sequenced and the sequence was verified to match the sequence in NM_000799 with the exception of a single base. Base 418 in the coding sequence (starting the numbering from the ATG) was C instead of G, changing amino acid 140 in the full length EPO sequence (*i.e.*, SEQ ID NO:6) starting from the first methionine from Arg to Gly. This is, however, normal sequence variation from the original sequence and present in most forms of erythropoietin.

Please replace the three paragraphs at p. 90, ll. 4-10 with the following amended three paragraphs:

This cDNA codes for the full length amino acid sequence of erythropoietin, which is below

MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENIT
TGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALL
VNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFR
KLFRVYSNFLRGKLKLYTGEACRTGDR ~~(SEQ ID NO: 10)~~ (SEQ ID NO:6).

The first 27 amino acid residues of ~~SEQ ID NO:10~~ SEQ ID NO:6 comprise a leader sequence.

Please replace the paragraph at p. 101, ll. 19-25 with the following amended paragraph:

The following are examples of constructs that were made: human EPO(hEPO)-6xHisTag-pCiNeo sequence (SEQ ID NO: 208); hEPO6xHisTag-A30N/H32T-pCiNeo (SEQ ID NO: 209); hEPO-6xHisTag-K45D-pCiNeo sequence (SEQ ID NO: 210); hEPO-6xHisTag-S100E-pCiNeo sequence (SEQ ID NO: 211); and hEPO-6xHisTag-K45D/S100E-pCiNeo sequence ~~(SEQ ID NO: 212)~~ (SEQ ID NO:5). The pCI-neo mammalian expression vector carries the human cytomegalovirus (CMV) immediate-early enhancer/promoter region to promote constitutive expression of cloned DNA inserts in mammalian cells.

Please replace the table at pp. 69-71 with the replacement table submitted herewith as Appendix A. The table is amended to replace the term "Guillian Barre" with the corrected term "Guillain Barre" (see specification as filed at p. 71, l. 13).

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of the claims:

1. (Withdrawn) A mutein recombinant tissue protective cytokine lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes, the cytokine comprising at least one responsive cellular protective activity selected from the group consisting of protecting, maintaining, enhancing or restoring the function or viability of a responsive mammalian cell, tissue or organ.

2. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising one or more altered amino acid residue between position 11 to 15 of SEQ ID NO:10 [SEQ ID NO:1], position 44 to 51 of SEQ ID NO 10 [SEQ ID NO:2], position 100-108 of SEQ ID NO [SEQ ID NO:3], or position 146-151 of SEQ ID NO 10 [SEQ ID NO:4].

3. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising an altered amino acid residue at one or more of the following positions of SEQ ID NO: 10: 7, 20, 21, 29, 33, 38, 42, 59, 63, 67, 70, 83, 96, 126, 142, 143, 152, 153, 155, 156, or 161.

4. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising the amino acid sequence of SEQ ID NO: 10 with one or more of the amino acid residue substitutions of SEQ ID NOs: 15-105 and 119.

5. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising the amino acid sequence of SEQ ID NO: 10 with a deletion of amino acid residues 44-49 of SEQ ID NO: 10.

6. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising, the amino acid sequence of SEQ ID NO: 10 with at least one of the following amino acid residue substitutions of SEQ ID NOs: 106-118.

7. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, further comprising a chemical modification of one or more amino acids.

8. (Withdrawn) The recombinant tissue protective cytokine of claim 7, wherein the chemical modification comprises altering the charge of the recombinant tissue protective cytokine.

9. (Withdrawn) The recombinant tissue protective cytokine of claim 8, wherein a positive or negative charge is chemically added to an amino acid residue where a charged amino acid residue is modified to an uncharged residue.

10. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is a human erythropoietin mutein.

11. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is a human phenylglyoxal erythropoietin mutein.

12. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein the responsive mammalian cell comprises a neuronal, muscle, heart, lung, liver, kidney, small intestine, adrenal cortex, adrenal medulla, capillary, endothelial, testis, ovary, endometrial, or stem cell.

13. (Withdrawn) The recombinant tissue protective cytokine responsive mammalian cell of any one of claims 1, 2, 3, 4, 5, or 6, comprising a photoreceptor, ganglion, bipolar, horizontal, amacrine, Mueller, myocardium, pace maker, sinoatrial node, sinus node, atrioventricular node, bundle of His, hepatocyte, stellate, Kupffer, mesangial, goblet, intestinal gland, enteral endocrine, glomerulosa, fasciculate, reticularis, chromaffin, pericyte,

Leydig, Sertoli, sperm, Graffian follicles, primordial follicles, endometrial stroma, and endometrial cell.

14. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is capable of traversing an endothelial cell barrier.

15. (Withdrawn) The recombinant tissue protective cytokine of claim 14, wherein the endothelial cell barrier comprises the blood-brain barrier, the blood-eye barrier, the blood testes barrier, the blood-ovary barrier, and the blood-uterus barrier.

16. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is selected from the group consisting of:

i. a cytokine having a reduced number or no sialic acid moieties;
ii. a cytokine having a reduced number or no N-linked or O-linked carbohydrates;
iii. a cytokine having at least a reduced carbohydrate content by virtue of treatment of native cytokine with at least one glycosidase;
iv. a cytokine having at least one or more oxidized carbohydrates;
v. a cytokine having at least one or more oxidized carbohydrates and is chemically reduced;
vi. a cytokine having at least one or more modified arginine residues;
vii. a cytokine having at least one or more modified lysine residues or a modification of the N-terminal amino group of a cytokine molecule;
viii. a cytokine having at least a modified tyrosine residue;
ix. a cytokine having at least a modified aspartic acid or glutamic acid residue;
x. a cytokine having at a modified tryptophan residue;
xi. a cytokine having at least one amino acid group removed;
xii. a cytokine having at least one opening of at least one of the cystine linkages in the cytokine molecule;
xiii. a truncated cytokine;
xiv. a cytokine having at least one polyethylene glycol molecule attached;
xv. a cytokine having at least one fatty acid attached;

xvi. a cytokine having a non-mammalian glycosylation pattern by virtue of the expression of a recombinant cytokine in non-mammalian cells; and
xvi. a cytokine having at least one histidine tagged amino acid to facilitate purification.

17. (Withdrawn) The recombinant tissue protective cytokine of claim 16 wherein said cytokine is an asialoerythropoietin.

18. (Withdrawn) The recombinant tissue protective cytokine of claim 17, wherein said asialoerythropoietin is human asialoerythropoietin.

19. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is hyposialylated or hypersialylated.

20. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 sialic acid moieties.

21. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises more than the fourteen sialic acid moieties present in native erythropoietin.

22. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin with no N-linked carbohydrates.

23. (Withdrawn) The recombinant tissue protective cytokine of claim 22, wherein said cytokine is an erythropoietin with no O-linked carbohydrates.

24. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is treated with at least one glycosidase.

25. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is periodate-oxidized erythropoietin.

26. (Withdrawn) The recombinant tissue protective cytokine of claim 25, wherein said periodate-oxidized erythropoietin is chemically reduced with sodium cyanoborohydride.

27. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises an R-glyoxal moiety on the one or more arginine residues, wherein R is aryl or alkyl moiety.

28. (Withdrawn) The recombinant tissue protective cytokine of claim 27, wherein said cytokine is phenylglyoxal-erythropoietin.

29. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin in which an arginine residue is modified by reaction with a vicinal diketone selected from the group consisting of 2,3-butanedione and cyclohexanedione.

30. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin in which an arginine residue is reacted with 3-deoxyglucosone.

31. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is a molecule having at least one biotinylated lysine or N-terminal amino group.

32. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is a glucitolyl lysine erythropoietin or fructosyl lysine erythropoietin.

33. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one carbamylated lysine residue.

34. (Withdrawn) The recombinant tissue protective cytokine of claim 33, wherein said carbamylated cytokine is comprised of alpha-N-carbamoylerythropoietin; N-epsilon-carbamoylerythropoietin; alpha-N-carbamoyl, N-epsilon-carbamoylerythropoietin; alpha-N-carbamoylasialoerythropoietin; N-epsilon-carbamoylasialoerythropoietin; alpha-N-carbamoyl, N-epsilon-carbamoylasialoerythropoietin; alpha-N-

carbamoylhyposialoerythropoietin; N-epsilon-carbamoylhyposialoerythropoietin; and alpha-N-carbamoyl, N-epsilon-carbamoylhyposialoerythropoietin.

35. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one acylated lysine residue.

36. (Withdrawn) The recombinant tissue protective cytokine of claim 35, wherein said cytokine comprises at least one acylated lysine residue.

37. (Withdrawn) The recombinant tissue protective cytokine of claim 36, wherein said cytokine comprises at least one acylated lysine residue.

38. (Withdrawn) The recombinant tissue protective cytokine of claim 37, wherein a said acetylated cytokine is comprised of alpha-N-acetylerythropoietin; N-epsilon-acetylerythropoietin; alpha-N-acetyl, N-epsilon-acetylerythropoietin; alpha-N-acetylasialoerythropoietin; N-epsilon-acetylasialoerythropoietin; alpha-N-acetyl, N-epsilon-acetylasialoerythropoietin; alpha-N-acetylhyposialoerythropoietin; N-epsilon-acetylhyposialoerythropoietin; and alpha-N-acetyl, N-epsilon-acetylhyposialoerythropoietin.

39. (Withdrawn) The recombinant tissue protective cytokine of claim 35, wherein a lysine residue of said cytokine is succinylated.

40. (Withdrawn) The recombinant tissue protective cytokine of claim 39, wherein said succinylated cytokine is comprised of alpha-N-succinylerythropoietin; N-epsilon-succinylerythropoietin; alpha-N-succinyl, N-epsilon-succinylerythropoietin; alpha-N-succinylasialoerythropoietin; N-epsilon-succinylasialoerythropoietin; alpha-N-succinyl, N-epsilon-succinylasialoerythropoietin; alpha-N-succinylhyposialoerythropoietin; N-epsilon-succinylhyposialoerythropoietin; and alpha-N-succinyl, N-epsilon-succinylhyposialoerythropoietin.

41. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one lysine residue modified by 2, 4, 6 trintrobenzenesulfonate sodium or another salt thereof.

42. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one nitrated or iodinated tyrosine residue.

43. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises an aspartic acid or glutamic acid residue that is reacted with a carbodiimide followed by reaction with an amine.

44. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein a said amine is glycinamide.

45. (Withdrawn) An isolated nucleic acid molecule that comprises a nucleotide sequence which encodes a polypeptide comprising the recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6.

46. (Withdrawn) A vector comprising a nucleic acid molecule of claim 45.

47. (Withdrawn) An expression vector comprising a nucleic acid molecule of claim 45 and at least one regulatory region operably linked to the nucleic acid molecule.

48. (Withdrawn) The vector of claim 46 or 47 that is a pCiNeo vector.

49. (Withdrawn) A genetically-engineered cell which comprises a nucleic acid molecule of claim 45.

50. (Withdrawn) A cell comprising the expression vector of claim 45.

51. (Withdrawn) A pharmaceutical composition comprising a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action,

hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes, the cytokine having at least one responsive cellular protective activity selected from the group consisting of protecting, maintaining, enhancing or restoring the function or viability of a responsive mammalian cell, tissue or organ.

52. (Withdrawn) The pharmaceutical composition of claim 51, formulated for oral, intranasal, or parenteral administration.

53. (Withdrawn) The pharmaceutical composition of claim 51, formulated as a perfusate solution.

54. (Currently amended) A method for protecting, maintaining or enhancing the viability of a responsive cell, a tissue comprising a responsive cell, or an organ comprising a responsive cell, wherein said cell, tissue or organ is isolated from a mammalian body, comprising exposing said cell, tissue or organ to an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine, wherein said mutein recombinant tissue protective cytokine

(a) comprises the amino acid sequence of SEQ ID NO:10 with a substitution of an amino acid residue at one of more of the following positions:
(i) 11 to 15 [SEQ ID NO:1];
(ii) 44 to 51 [SEQ ID NO:2];
(iii) 100 to 108 [SEQ ID NO:3]; or
(iv) 146 to 151 [SEQ ID NO:4];

(b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay; and

(c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay. ~~, wherein the viability of the cell, tissue, or organ is protected, maintained, or enhanced.~~

55. (Currently amended) The method of claim 54, wherein the mutein recombinant tissue protective cytokine ~~does not affect bone marrow~~ is nonerythropoietic.

56. (Currently amended) ~~A~~ The method of claim 54, wherein the mutein ~~for protecting, maintaining or enhancing the viability of a responsive cell, a tissue comprising a responsive cell, or an organ comprising a responsive cell, isolated from a mammalian body comprising exposing said cell, tissue or organ to a pharmaceutical composition comprising a~~ recombinant tissue protective cytokine ~~of any one of claims 1, 2, 3, 4, 5, or 6, that~~ lacks at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes~~, wherein the viability of the cell, tissue, or organ is protected, maintained, or enhanced~~.

57. (Currently amended) A method for ~~the~~ protecting against ~~and~~ or preventing a tissue injury ~~as well as~~ or restoring ~~and~~ or rejuvenating tissue ~~and~~ or tissue function in a mammal, comprising exposing said tissue to ~~a~~ an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine ~~of any one of claims 1, 2, 3, 4, 5, or 6,~~ wherein said mutein recombinant tissue protective cytokine

(a) comprises the amino acid sequence of SEQ ID NO:10 with a substitution of an amino acid residue at one of more of the following positions:
(i) 11 to 15 [SEQ ID NO:1];
(ii) 44 to 51 [SEQ ID NO:2];
(iii) 100 to 108 [SEQ ID NO:3]; or
(iv) 146 to 151 [SEQ ID NO:4];

(b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay; and

(c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay. ~~that lacks at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes, wherein the method results in protection against and prevention of a tissue injury as well as restoration and rejuvenation of tissue and tissue function in a mammal.~~

58. (Currently amended) The method of claim 57, wherein the mammal has or is at risk for cognitive dysfunction, a seizure disorder, chronic seizure disorder, epilepsy, convulsions, nerve root compression, myotonic dystrophy, muscular dystrophy, multiple sclerosis, stroke, hypotension, cardiac arrest, central nervous system injury, neuronal loss, ischemia, subdural hematoma, subarachnoid bleeds, aneurysm, aneurysmal bleeds, myocardial infarction, inflammation, age-related loss of cognitive function, radiation damage, chemotherapy damage, radiotherapy damage, whole brain irradiation damage, cerebral palsy, cerebral supranuclear palsy, progressive supranuclear palsy, neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Huntington's disease, Tourette's syndrome, Leigh disease, Guillain Barre, dementia, AIDS dementia, senile dementia, Lewy body dementia, memory loss, amyotrophic lateral sclerosis, alcoholism, a neuropsychiatric or neuropsychological disorder, mood disorder, anxiety disorder, anxiety, schizophrenia, schizoaffective disorder, obsessive-compulsive disorder, panic disorder, uni-polar affective disorder, depression, major depressive disorder, dysthymic disorder, mania, bi-polar affective disorder, attention deficit disorder, attention deficit hyperactivity disorder, autism, a prion disease, Creutzfeldt-Jakob ~~Creutzfeld-Jakob~~ disease, Friedrich's ataxia, Wilson's disease, trauma, concussive injury, brain or spinal cord trauma or ischemia, heart-lung bypass, neurological defects from heart-lung bypass, post-operative cognitive dysfunction, embolic injury, hypoxia, mitochondrial dysfunction, abdominal aortic surgery, heart injury, myocardium injury, heart trauma, chronic heart failure, eye tissue damage, macular degeneration, diabetic neuropathy, diabetic retinopathy, glaucoma, retinal ischemia, ~~or~~ retinal trauma, retinitis pigmentosa, optic nerve damage, retinal detachment, arteriosclerotic retinopathy, hypertensive retinopathy, retinal artery blockage, retinal vein blockage, hypotension, a condition associated with hypoglycemia or diabetes, diabetes mellitus, nephrotic symptoms, acute renal failure, or hepatitis.

59. (Withdrawn) A method for facilitating the transcytosis of a molecule across an endothelial cell barrier in a mammal comprising administration to said mammal a composition comprising said molecule in association with a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, increasing blood pressure, hyperactivating platelets, and increasing production of thrombocytes.

60. (Withdrawn) The method of claim 59, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.

61. (Withdrawn) The method of claim 59, wherein said endothelial cell barrier is selected from the group consisting of the blood-brain barrier, the blood-eye barrier, the blood-testis barrier, the blood-ovary barrier, the blood-heart barrier, the blood-kidney barrier, and the blood-placenta barrier.

62. (Withdrawn) The method of claim 59, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, an antiviral agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, a marker, or an anti-cancer drug.

63. (Withdrawn) A composition for transporting a molecule via transcytosis across an endothelial cell barrier comprising said molecule in association with a recombinant tissue protective cytokine, of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes.

64. (Withdrawn) The composition of claim 63, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.

65. (Withdrawn) The composition of claim 63, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, a marker, or an anti-cancer drug.

66. (Withdrawn) Use of an recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing

hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes.

67. (Withdrawn) The use of claim 66, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.

68. (Withdrawn) The use of claim 66, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, or a marker, or an anti-cancer drug.

69. (Currently amended) The method of claim 57, wherein the mutein recombinant tissue protective cytokine is administered to the mammal prior to a surgical procedure.

70. (Previously presented) The method of claim 69, wherein the surgical procedure is cardiopulmonary bypass surgery.

71. (New) A method for protecting, maintaining or enhancing the viability of a responsive cell, a tissue comprising a responsive cell, or an organ comprising a responsive cell, wherein said cell, tissue or organ is isolated from a mammalian body, comprising exposing said cell, tissue or organ to an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine, wherein said mutein recombinant tissue protective cytokine

(a) comprises an amino acid sequence comprising amino acid positions 1 to 10, 16 to 43, 52 to 99, 109 to 145, and 152 to 166 of SEQ ID NO:10;

(b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay; and

(c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay.

72. (New) A method for protecting against or preventing a tissue injury or restoring or rejuvenating tissue or tissue function in a mammal comprising exposing said tissue to an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine, wherein said mutein recombinant tissue protective cytokine

(a) comprises an amino acid sequence comprising amino acid positions 1 to 10, 16 to 43, 52 to 99, 109 to 145, and 152 to 166 of SEQ ID NO:10;

(b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay; and

(c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay.

73. (New) The method of claim 72, wherein the mammal has or is at risk for cognitive dysfunction, a seizure disorder, chronic seizure disorder, epilepsy, convulsions, nerve root compression, myotonic dystrophy, muscular dystrophy, multiple sclerosis, stroke, hypotension, cardiac arrest, central nervous system injury, neuronal loss, ischemia, subdural hematoma, subarachnoid bleeds, aneurysm, aneurysmal bleeds, myocardial infarction, inflammation, age-related loss of cognitive function, radiation damage, chemotherapy damage, radiotherapy damage, whole brain irradiation damage, cerebral palsy, cerebral supranuclear palsy, progressive supranuclear palsy, neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Huntington's disease, Tourette's syndrome, Leigh disease, Guillain Barre, dementia, AIDS dementia, senile dementia, Lewy body dementia, memory loss, amyotrophic lateral sclerosis, alcoholism, a neuropsychiatric or neuropsychological disorder, mood disorder, anxiety disorder, anxiety, schizophrenia, schizoaffective disorder, obsessive-compulsive disorder, panic disorder, uni-polar affective disorder, depression, major depressive disorder, dysthymic disorder, mania, bi-polar affective disorder, attention deficit disorder, attention deficit hyperactivity disorder, autism, a prion disease, Creutzfeldt-Jakob disease, Friedrich's ataxia, Wilson's disease, trauma, concussive injury, brain or spinal cord trauma or ischemia, heart-lung bypass, neurological defects from heart-lung bypass, post-operative cognitive dysfunction, embolic injury, hypoxia, mitochondrial dysfunction,

abdominal aortic surgery, heart injury, myocardium injury, heart trauma, chronic heart failure, eye tissue damage, macular degeneration, diabetic neuropathy, diabetic retinopathy, glaucoma, retinal ischemia, retinal trauma, retinitis pigmentosa, optic nerve damage, retinal detachment, arteriosclerotic retinopathy, hypertensive retinopathy, retinal artery blockage, retinal vein blockage, hypotension, a condition associated with hypoglycemia or diabetes, diabetes mellitus, nephrotic symptoms, acute renal failure, or hepatitis.

REMARKS

By this amendment, the specification has been amended to correct minor clerical errors and to incorporate the Substitute Sequence Listing submitted herewith.

In particular, the paragraph at p. 20, ll. 3-17 of the specification has been amended to correct the spelling of the term "Creutzfeldt-Jakob"; the paragraph at p. 47, ll. 11-22 of the specification has been amended for clarity and to correct spelling errors; the paragraph at p. 68, ll. 6-25 of the specification has been amended to correct the spelling of the term "Friedrich's"; and the table at p. 71, l. 13 has been amended to correct the spelling of the term "Guillain Barre."

In addition, the paragraphs at p. 9, l. 27 to p. 10, l. 5 and at p. 42, ll. 1-23 of the specification have been amended to correct typographical errors and to update the reference to the U.S. patent application that was identified as Attorney Docket No. KW00-009C02-US to state that it has been accorded Application Serial No. 10/188,905. A copy of the filing receipt for Application Serial No. 10/188,905 is attached as Exhibit A. As such, no new matter has been added with this amendment. See M.P.E.P. 608.01(p).

The specification at p. 42, ll. 1-23 has also been amended to add the language "in which *in vivo* biological activity was determined by the polycythemic mouse bioassay." This amendment is supported by the application as originally filed at p. 42, ll. 10-11, which incorporates by reference Satake *et al*; 1990, *Biochim. Biophys. Acta* 1038:125-9 in its entirety for its description of molecular biological techniques for generating erythropoietin derivatives and testing them for *in vivo* biological activity of erythrocytes (*i.e.*, erythropoietic activity) using the polycythemic mouse bioassay. See Satake, page 126, col. 2: "in vivo biological activity was determined by the exhypoxic polycythemic mouse bioassay," citing Cotes and Bangham, 1961, Nature 191:1065-1067 (reference C102 of record in the subject application). As such, no new matter has been added with this amendment. See M.P.E.P. 608.01(p).

Lastly, sequence identifiers were inserted into the paragraphs at p. 39, l. 8 to p. 40, l. 14 and p. 89, ll. 14-20. In the paragraphs at p. 90, ll. 4-10, the sequence identifier for full-length erythropoietin (EPO) has been amended from SEQ ID NO:10 to SEQ ID NO:213.

Claims 1-70 were pending in the instant application. Claims 1-53, 59-68, and 69-70 are withdrawn from consideration. By this amendment, claims 54-58 are amended and new claims 71-73 are added, for the reasons discussed below.

Claims 54 and 57 have been amended to specify that the mutein recombinant tissue protective cytokine (alternatively referred to herein as "EPO mutein") comprises the amino acid sequence of SEQ ID NO:10 with a substitution of an amino acid residue at one or more of the following positions: (i) 11 to 15 [SEQ ID NO:1]; (ii) 44 to 51 [SEQ ID NO:2]; (iii) 100 to 108 [SEQ ID NO:3]; or (iv) 146 to 151 [SEQ ID NO:4]. Support for this amendment may be found in the claims and specification as filed, *inter alia*, at p. 4, ll. 20 to p. 5, l. 4; p. 6, ll. 15-16; p. 6, l. 22 to p. 9, l. 20; p. 40, ll. 3-7; Example 3 starting at p. 89, particularly p. 90, ll. 4-10 and p. 101, l. 19 to p. 102, l. 24; Example 15 at pp. 124-125; Example 16 at pp. 125-126; and Example 18 at pp. 128-129; the Substitute Sequence Listing for the corresponding sequences referred to in the aforementioned pages; and claim 2 as originally filed. Claims 54 and 57 have also been amended to recite specific tests for determining erythropoietic and tissue protective activity. In particular, the recited muteins have: (i) a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay, and (ii) tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay. Support for this amendment may be found in the specification *inter alia* at p. 42, paragraph beginning at l. 1 as amended herein; Example 3 at p. 103, l. 18 to p. 104, l. 3; and Example 15 at pp. 124-125. In addition, claims 54 and 57 have been amended to recite that "an effective amount" of a pharmaceutical composition is administered. Support for this amendment may be found in the specification, *e.g.*, at p. 19, ll. 1-19.

Claim 58 has been amended to add the term "is at risk for." Support for this amendment may be found in the specification at, *inter alia*, p. 20, ll. 3-8; p. 24, ll. 16-19; p. 31, ll. 26-30; p. 64, ll. 13-15 and ll. 29-32; p. 79, ll. 19-25; and p. 80, ll. 6-15. The listing of indications in claim 58 has been amended to more distinctly point out the invention. Support for this amendment may be found in the specification, *e.g.*, at p. 20, ll. 3-17; p. 31, ll. 21-30; p. 66, l. 25 to p. 68, l. 32; the Table at pp. 69-71; and p. 79, ll. 14-25. Claim 58 has also been amended to correct the spelling of Creutzfeldt-Jakob disease.

Claims 54 and 57 have also been amended to correct minor typographical and/or grammatical errors and to clarify the invention. Claim 56 has been amended to depend from claim 54. Claim 69 has been amended so that it is consistent with antecedent claim 57 as amended.

Support for new claims 71-73 may be found in the specification and claims as filed, *inter alia*, at p. 4, ll. 20 to p. 5, l. 4; p. 6, ll. 15-16; p. 6, l. 22 to p. 9, l. 20; p. 19, ll. 1-19; p. 20, ll. 3-17; p. 24, ll. 16-19; p. 31, ll. 21-30; p. 40, ll. 3-7; p. 42, paragraph beginning at l. 1 as amended herein; p. 64, ll. 13-15 and ll. 29-32; p. 66, l. 25 to p. 68, l. 32; the Table at pp. 69-71; p. 79, ll. 14-25; p. 80, ll. 6-15; Example 3 starting at p. 89, particularly p. 90, ll. 4-10 and p. 101, l. 19 to p. 102, l. 24; Example 15 at pp. 124-125; Example 16 at pp. 125-126; and Example 18 at pp. 128-129; the Substitute Sequence Listing for the corresponding sequences referred to in the aforementioned pages; and claim 2 as originally filed.

No new matter has been added by this amendment. Thus, claims 1-73 will be pending upon entry of the present amendment, and claims 54-58 and 69-73 will be under examination.

I. STATEMENT OF THE SUBSTANCE OF THE INTERVIEW

Applicants thank Primary Examiner Aditi Dutt and Supervisory Patent Examiner Jeffrey Stucker for the courtesies extended during the interview of February 6, 2008 at the United States Patent and Trademark Office ("the Interview"). Also present at the Interview were Drs. Anthony Cerami and Michael Brines, two of the inventors of the instant application, Frederick J. Hamble, Esq., of Warren Pharmaceuticals, Inc., Mary Catherine DiNunzio, Esq., of H. Lundbeck A/S, and Applicants' representatives Laura A. Coruzzi, Esq., Eileen E. Falvey, Esq., and Tracy J. LaGrassa, Ph.D., of Jones Day.

During the Interview an overview of the invention was presented. In summary, the inventors described their discovery that EPO provides tissue protective activity via a pathway distinct from the pathway it uses for erythropoiesis, *i.e.*, red blood cell production. EPO exerts its erythropoietic effect via an EpoR homodimer (the "Classical EPO Receptor"), whereas EPO's tissue protective activity is mediated through its interaction with a different receptor, referred to herein as the "Tissue Protective Receptor Complex," which is a

heteromer of EpoR and the beta common receptor (βcR). EPO signaling through the Classical EPO Receptor (present predominantly on blood forming cells) elicits an increase in erythrocytes, platelets, and blood pressure. In contrast, activation by EPO of the Tissue Protective Receptor Complex (present on many tissues) leads to a wider range of tissue protective effects. Based on the inventors' discovery that EPO can cross tight endothelial cell barriers, they found that exogenously administered EPO is capable of conferring tissue protection on any tissue that expresses the Tissue Protective Receptor Complex. Therefore, as a tissue protective cytokine, EPO counteracts the tissue damage at the root of a wide variety of diseases and disorders. For example, the inventors found that EPO protects against tissue damage caused by the pro-inflammatory cytokine TNFα, reduces the extent of TNFα-induced damage, and promotes healing and regeneration of affected tissues. Thus, the ability of EPO to prevent cell death and tissue damage that is common to many disease conditions demonstrates the widespread therapeutic implications of EPO and EPO muteins.

The inventors found that modified EPOs that cannot bind to the Classical EPO Receptor and stimulate red blood cell production were still effective in their tissue protective functions. Such modified EPOs have the benefit of providing tissue protection without the effect of increasing erythropoiesis, and therefore are suitable for high dose administration or chronic administration often required for tissue protection – for example, for administration to stroke victims – while avoiding the risk of thrombosis associated with administration of wild-type EPO. This concept was demonstrated with *in vitro* and *in vivo* experiments that showed that EPO muteins, particularly those with amino acid substitutions in the domains needed for binding to the Classical EPO Receptor, and EPOs in which the amino acid side chains have been masked by chemical modifications, are both nonerythropoietic and tissue protective in various animal disease models.

The outstanding rejections under 35 U.S.C. § 112, first paragraph, for scope of enablement, made in the Office Action dated December 31, 2007 (the "Office Action") were discussed. Because the Tissue Protective Receptor Complex is present on a large variety of tissues, and EPO muteins with reduced erythropoietic function are tissue protective in a broad range of diseases and injuries, EPO muteins are able to confer tissue protection in a broad range of tissues and for a broad range of diseases and injuries. The possibility of amending

the claims to recite EPOs with mutations in specific regions that affect binding to the classical EPO-R homodimers was discussed.

In view of this discussion, the Examiners indicated that claims reciting EPO muteins having the structural characteristics of having mutations in the regions needed for receptor homodimer binding but not in the remainder of the molecule and the functional characteristics of exhibiting tissue protective activity would be considered if excessive searches were not required.

The Examiners also indicated that they might reconsider the species election set forth in the restriction requirement, mailed June 20, 2006. Specifically, the Examiners indicated that they would consider examining the claims with respect to a greater number of EPO muteins, each mutein capable of binding the Tissue Protective Receptor Complex but not the Classical EPO Receptor, as long as excessive further searches of EPO sequences would not be required.

Applicants also presented results of experiments to support enablement commensurate with the full breadth of the limitation "protecting against and preventing a tissue injury" as well as "restoring and rejuvenating tissue and tissue function in a mammal." The Examiners indicated that the evidence and arguments would be considered if presented.

This Amendment, the evidence presented in the Declaration of Dr. Michael L. Brines, M.D., Ph.D. (the "Brines Declaration") submitted herewith, and the remarks herein reflect the discussion during the Interview.

II. SUBSTITUTE SEQUENCE LISTING

A Substitute Sequence Listing is submitted herewith to correct the Sequence Listing, which lists sequences of the full-length EPO precursor, which includes a leader sequence, whereas the specification discloses amino acid positions of the mature EPO protein, which lacks the leader sequence.

The Substitute Sequence Listing is amended so that SEQ ID NOs:4, 10, and 15-119 represents mature EPO sequences rather than full-length EPO sequences. SEQ ID NO:6 in the Substitute Sequence Listing now represents the full-length EPO precursor.

No new matter is added by this Substitute Sequence Listing, which is supported by the specification as filed. The specification as filed discloses that the first 27 amino acid residues of full-length EPO is a leader sequence (see p. 90, ll. 4-10) and it was well known in the art that mature EPO is produced by the removal of this 27-amino acid leader sequence. See, *e.g.*, Jacobs K *et al.* 1985. "Isolation and characterization of genomic and cDNA clones of human erythropoietin," *Nature* 313(6005):806-810; reference C173 in the Supplemental Information Disclosure Statement submitted concurrently herewith. The specification, however, refers to amino acid positions in mature EPO, but misidentifies them with sequence identifiers that represent full-length EPO (see, *e.g.*, p. 4, ll. 24-25; p. 90, ll. 4-10; and SEQ ID NO:10 of the specification as filed, which states that the sequence TKVNFYAW is at amino acid positions 44-51, but shows that this sequence is located in full-length EPO at positions 71-78). Thus, one of skill in the art reading the specification would understand that the EPO amino acid positions in the specification refer to mature EPO. Therefore, no new matter has been added with the Substitute Sequence Listing.

The Substitute Sequence Listing also contains a correction to the listing of the sequences in response to the Examiner's objection that SEQ ID NO:62 and SEQ ID NO:5 are the same. The sequence identified by SEQ ID NO:5 has been replaced with the sequence formerly identified as SEQ ID NO:212. Applicants note that SEQ ID NO:6 and SEQ ID NO:44 are also identical in the Sequence Listing as filed. In order to correct this duplication, the sequence identified by SEQ ID NO:6 has been replaced with the sequence formerly identified as SEQ ID NO:10 (full-length EPO) in the Substitute Sequence Listing.

III. REQUEST FOR RECONSIDERATION OF RESTRICTION REQUIREMENT AND SPECIES ELECTION

As currently amended, claims 54 and 57 and their dependent claims thereon are limited with respect to the number of mutein recombinant tissue protective cytokines (hereinafter referred to as "EPO muteins") that fall within their scope. In view of this amendment, Applicants respectfully request that the Examiner reconsider the finality of the restriction requirement and the species election of a single EPO point mutation. In particular, Applicants request consideration of the full scope of claims 54-58 as amended, and request

that the Examiner withdraw the objection to claims 56-58 as allegedly reciting non-elected subject matter.

Applicants also traverse the Examiner's withdrawal of claims 69 and 70, and request reconsideration of their withdrawal. Applicants submit that these claims are within the scope of their antecedent claim 57. Specifically, claim 57 as currently amended recites a method for "protecting against or preventing a tissue injury" using an EPO mutein. Claims 69 and 70 recite that the EPO mutein is administered before a surgical procedure, which is a specific embodiment of the method of claim 57. See specification *inter alia* at p. 31, ll. 21-30 and p. 64, ll. 17-32. Accordingly, the subject matter of claims 69 and 70 is within the scope of claim 57 and therefore within the scope of the elected subject matter. As such, it is believed that claims 69 and 70 would not require additional searches. Applicants therefore respectfully request that the Examiner consider claims 69 and 70.

IV. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, FOR LACK OF ENABLEMENT, SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. In particular, the Examiner contends that the specification does not reasonably provide enablement across the full scope of the claims. The Examiner acknowledges that the art recognizes protective effects of four different EPO muteins in ischemic injury models and certain inflammatory conditions, but contends that the art and the specification fail to provide guidance or sufficient scientific basis to use any tissue protective cytokine for any tissue injury (Office Action, p. 5). The Examiner concludes that undue experimentation would be required to practice the claimed invention based on the application of the *Wands* factors: "breadth of the claims encompassing the use of molecules with no precise structural requirements, the lack of adequate guidance . . . supporting a therapeutic effect of EPO molecules on the broadly claimed chronic and/or degenerative diseases or disorders, or guidance on their use, the unpredictability in the art . . . and the complex nature of the invention" (Office Action, ¶ bridging pp. 8-9).

Applicants believe that amended claims 54-58 and new claims 71-73 are enabled over their entire scope. Each of these claims recites a method comprising exposing cells, tissue, or organs to a particular EPO mutein. One of skill in the art can readily practice the claimed

invention over its entire scope without undue experimentation. The claims recite a defined class of EPO muteins with amino acid substitutions at a limited number of amino acid residues. The claims further provide a clear-cut test that can be used by the skilled artisan to determine whether any particular EPO mutein within this defined class has the required activity, *i.e.*, tissue protection but reduced erythropoietin activity. Thus, a skilled person can readily determined the limited number of EPO muteins encompassed buy the claims, and use them to practice the invention without undue experimentation.

1. EPO muteins

Claims 54-58 have been amended to recite methods using specific EPO muteins with amino acid substitutions in four regions that affect binding to the Classical EPO Receptor, *i.e.*, amino acids at positions 11 to 15 of SEQ ID NO:10 [SEQ ID NO:1]; 44 to 51 of SEQ ID NO:10 [SEQ ID NO:2]; 100 to 108 of SEQ ID NO:10 [SEQ ID NO:3]; and 146 to 151 of SEQ ID NO:10 [SEQ ID NO:4] (see specification at, *e.g.*, p. 40, ll. 2-7). Because these EPO muteins have amino acid substitutions in regions that affect binding to the Classical EPO Receptor, they have reduced erythropoietic activity. However, since the amino acids in these regions do not affect binding to the Tissue Protective Receptor Complex, these EPO muteins maintain their tissue protective function. See Brines Declaration, ¶¶ 23-24.

The claims have also been amended to recite specific, art-recognized tests that require that the EPO muteins have reduced erythropoietic activity yet maintain tissue protective activity. In particular, the claims require that the EPO muteins have (i) a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay and (ii) tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay.

As such, the claims as amended recite a limited class of EPO muteins having structural and functional features necessary to provide tissue protection while having a reduced ability to stimulate erythropoiesis and are not directed to <u>any</u> tissue protective cytokine.

The amended and new claims are enabled by the specification's extensive teaching of methods to make the claimed EPO muteins and test them for erythropoietic and tissue protective activity. See Brines Declaration, ¶¶ 5 and 18-22. Additional such assays were well-known in the art as of the filing date of the instant application. See Brines Declaration, *id.* Indeed, several such muteins have been made and tested for erythropoietic activity and tissue protective activity in accordance with these teachings, as demonstrated by the working examples of the specification and experiments conducted after the filing date of the instant application (see explained in the Brines Declaration, ¶¶ 5-12), as described below.

Amino acids 100-108. As examples of EPO muteins with amino acid substitutions at positions 100 to 108 of SEQ ID NO:10 [SEQ ID NO:3], the specification provides working examples that show that EPO muteins with substitutions in this region are tissue protective and have reduced erythropoietic activity compared to wild-type recombinant EPO. For example, the EPO muteins S100E and R103E have reduced erythropoietic activity (see specification, Example 17 at p. 126, l. 12 to p. 128, l. 13) but are tissue protective. In particular, the specification teaches that S100E maintains the viability of neuroblastoma cells against rotenone in an *in vitro* assay (Example 3 at page 102, ll. 5-23). The specification further teaches that S100E is protective in well-established assays for apoptosis, for example, protection of P19 cells from apoptosis following serum withdrawal (Example 15 at p. 124, l. 1 to p. 125, l. 2), protection of primary hippocampal neuron cell cultures from NMDA-induced cell death (Example 14 at p. 122, ll. 30 to p. 123, l. 30), and protection of neuronal-like cells from cell death upon withdrawal of nerve growth factor (Example 16 at p. 125, l. 5 to p. 126, l. 6). See also Brines Declaration, ¶¶ 8-9. In addition, the mutein R103E has been shown to reduce NMDA-induced apoptosis of rat hippocampal neurons *in vitro*. See Brines Declaration, ¶ 9. Thus, in well-established *in vitro* assays for apoptosis using different cell types, muteins substituted in amino acid positions 100-108 were shown to be cell-protective.

These *in vitro* results have been corroborated by *in vivo* studies. Example 12 of the specification shows that the S100E mutein reduces functional deficits in motor neurological function in an animal model of traumatic spinal cord injury (specification at p. 115, ll. 21-30 and p. 116, l. 20 to p. 117, l. 5). The S100E mutein and the R103E mutein are tissue protective in retina, shown using an animal model of glaucoma. See Example 18 of the

specification at p. 128, l. 17 to p. 129, l. 8. See also Brines Declaration, ¶ 10. In a more recent study, the mutein S100E was demonstrated to improve neurological function after stroke. In another experiment, S100E and R103E were found to prevent injury that was caused by sciatic nerve compression in rats. See Brines Declaration, ¶¶ 11-12.

The specification also teaches that the double substitution EPO mutein K45D/S100E is tissue-protective in an *in vivo* animal model of glaucoma. See Example 18 of the specification at p. 128, l. 17 to p. 129, l. 8. Moreover, the double substitution mutein K45D/S100E has been demonstrated to prevent injury due to sciatic nerve compression in rats. See Brines Declaration, ¶ 12.

Amino acids 146-151. As an example of an EPO mutein with amino acid substitutions at positions 146 to 151 of SEQ ID NO:10 [SEQ ID NO:4], the specification teaches that the EPO mutein R150E prevents NMDA-induced cell death in primary hippocampal neuron cell cultures. See specification, Example 14 at p. 122, ll. 30 to p. 123, l. 30. Moreover, in a subsequent study, R150E was found to prevent P19 cell apoptosis. See Brines Declaration, ¶ 9. Again, these *in vitro* results were confirmed *in vivo*. For instance, the specification provides a working example to demonstrate that R150E is tissue protective in retina in an *in vivo* animal model of glaucoma. See specification, Example 18 at p. 128, l. 17 to p. 129, l. 8.

Chemically-modified amino acids. EPO muteins with amino acid substitutions in four regions that affect binding to the Classical EPO Receptor have reduced erythropoietic activity but maintain their tissue protective function. A similar effect is achieved when the charge of amino acids in these four regions is altered by chemical modification. See Brines Declaration, ¶ 13. Indeed, the specification teaches several chemical modifications of the EPO molecule that result in EPOs with tissue protective activity but reduced erythropoietic activity. Such modifications include modification of arginine residues with a vicinal diketone (*e.g.*, cyclohexanedione) or R-glyoxal (*e.g.*, phenylglyoxal), modification of tyrosine residues by nitration (using, *e.g.*, a trinitrophenyl compound) or iodination, modification of lysine residues by, *e.g.*, carbamylation, guanidination, or carboxymethylation, and oxidation of tryptophan residues by succinylation. See specification at p. 52, l. 1 to p. 53, l. 25; p. 54, ll. 8-10; p. 55, ll. 13-18; p. 109, ll. 23-28; Example 4 at p. 104, ll. 11-22 and p. 105, l. 23 to p.

107, l. 10; Example 5 at p. 108, l. 19 to p. 109, l. 16; Example 6 starting at p. 111; Example 12 at p. 117, l. 6 to p. 118, l. 15.

EPOs with chemically-modified lysines (EPOs that have been carbamylated, carboxymethylated, PEGylated, carbamylated and PEGylated, or succinylated), arginines (by cyclohexanedionation and phenylglyoxalation), glutamate/aspartate (EDC-ethanolamination), and tyrosines (trinitrophenylation), and EPOs in which the disulfides are reduced by iodoacetamidation, have been shown to be nonerythropoietic yet tissue protective in various tissues and disease or injury conditions. See Brines Declaration, ¶¶ 13-17.

Accordingly, if an EPO with a chemically-modified arginine, for example, in a domain that affects Classical EPO Receptor binding (*i.e.*, a chemical modification of the arginine at position 14, 103 or 150) retains its tissue protective function, it would follow that an EPO having a single substitution of an arginine at that same position would also be tissue protective. This is indeed the case, as described *supra* for the R103E and R150E muteins. Therefore, the experiments showing a tissue protective function for EPOs with chemically-modified amino acids at particular positions indicate that EPOs with mutations in amino acids at those positions – in particular, amino acids 11-15 of SEQ ID NO:10 (VLQ**RY**), amino acids 44-51 (T**K**VNF**Y**A**W**), 100-108 (SGL**R**SLTTL), and 146-151 (SNFL**R**G) (residues that have been modified by chemical modification are shown in bold/underline) – are also tissue protective.

2. Protection or Rejuvenation of Tissues

The Examiner acknowledges that routine technology and bioassays can be used to make the claimed EPO muteins, but contends that the testing of numerous mutants for tissue protection would require undue experimentation, because tissue responsiveness will vary depending on the mutein, different mutein doses must be tested, and *in vitro* testing does not parallel the *in vivo* protection, wherein the injury can arise due to various causes (Office Action, p. 8). Applicants respectfully submit that practicing the claimed invention does not require undue experimentation.

The methods of the present claims are used to prevent and treat conditions that result from cell death and subsequent tissue damage induced by inflammation and oxidative

damage. Because the Tissue Protective Receptor Complex is present on a large variety of tissues, and EPO muteins with reduced erythropoietic function are tissue protective in a broad range of diseases and injuries, EPO muteins are able to confer tissue protection in a broad range of tissues and for a broad range of diseases and injuries. Thus, an EPO mutein found to have tissue protective activity in the *in vitro* or *in vivo* assays recited in the claims can be used in the claimed methods regardless of the source of the injury, other symptoms of the disease or condition, and the cell type affected. See Brines Declaration, ¶¶ 23-28.

Therefore, the skilled artisan would be able to practice the claimed invention with a limited amount of experimentation using routine, art-accepted methods. This amount of experimentation is well within the legal standard, which does not preclude a certain amount of experimentation and unpredictability of the results. *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988); *In re Angstadt*, 190 U.S.P.Q. 214, 219 (C.C.P.A. 1976); *In re Neuberger & Rabbitts,* 2002 WL 33952578 (B.P.A.I. 2002)(Board reversed Examiner's non-enablement rejection of claims to a biotechnological invention because "a requirement for certainty would be incompatible with any amount of experimentation and therefore incompatible with the standard of enablement." *Id.* at 3). All that is required is a reasonable amount of guidance with respect to the direction of the experimentation; reasonable certainty with regard to the *outcome* of the experimentation is *not* required.

Moreover, the skilled artisan would understand that a certain amount of experimentation and optimization is required to determine the efficacy (whether using a cell line or experimental animal or in a patient) of a particular EPO mutein. Indeed, the optimal dosage *for any* pharmaceutical composition must be decided according to the judgment of the practitioner and each patient's circumstances, and the dose is determined based not only on the particular EPO mutein but also on the condition or disease to be treated, the body mass of the patient, the route of administration, *etc.* See specification at p. 77, ll. 8-20. As stated in *In re Brana*, 5 F.3d 1557, 34 U.S.P.Q.2d 1437, 1442 (Fed. Cir. 1995), the testing for the full safety and *effectiveness* of a product is more properly left to the Food and Drug Administration and the requirements under the law for obtaining a patent should not be confused with the requirements for obtaining government approval to market a particular drug or therapeutic method for public use.

Thus, based on the applicable case law and the teachings of the specification, Applicants do not believe that the need to determine the optimal dosage of a mutein is a proper basis for an allegation of undue experimentation.

Furthermore, Applicants also disagree with the Examiner's contention that *in vitro* testing does not parallel the *in vivo* protection. Indeed, the tissue protection obtained in *in vitro* and *in vivo* assays with specific EPO muteins described *supra* provides ample evidence to the contrary. Tissue protection *in vitro* is predictive of tissue protection *in vivo*, as demonstrated by the working examples of the instant specification and explained in the Brines Declaration. See Brines Declaration, ¶¶ 18-20.

Finally, the Examiner acknowledges that EPO muteins spare neuronal tissue loss due to injury and reduce cell death, but states that it would be necessary to "implicate EPO as being able to induce *new cellular growth*, which would be necessary to restore or rejuvenate tissue" (Office Action, ¶ bridging pp. 5-6; emphasis added). Applicants respectfully disagree that a demonstration that EPO can induce new cellular growth is required to "restore" or "rejuvenate" tissue.

The term "restore" in the claims is understood to mean (1): give back, return; and (2): to put or bring back into existence or use (definitions (1) and (2) from Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, p. 1005, entry for "restore" (Exhibit B)). In the context of restoring function, meanings (1) and (2) are appropriate where a mutein may be used to "give back" function to a cell, tissue, or organ that had lost function, *e.g.*, as a result of injury or trauma, or where a mutein may be used to put a cell, tissue, or organ "back into use." By analogy, a work of art or a house may be "restored," but it is never fully returned to its original state or condition. Likewise, Applicants submit that the ordinary meaning of the term "restore," as it is used in the instant claims, does not require new cell growth or even that the claimed cells, tissues, and organs are fully returned to their original state or regain full function.

Likewise, "rejuvenate" means: (a) to make young or youthful again: reinvigorate; (b): to restore to an original or new state (definition (1) from Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, p. 994, entry for "rejuvenate"

(Exhibit B)). In the context of rejuvenating tissue or tissue function, this definition is appropriate where a mutein may be used to "reinvigorate" a tissue that had lost function, for example, as a result of injury or trauma, or where a mutein may be used to "restore" (as defined in the preceding paragraph) a tissue – not necessarily to its original state, but to *a new, functional state*. As such, Applicants submit that the ordinary meaning of the term "rejuvenate," as it is used in the instant claims, does not require new cell growth but only that the function of the tissue or organ is restored.

These definitions of restore and rejuvenate are consistent with the teachings of the specification. For example, the specification provides an experiment in which mice were subjected to a brain trauma that impairs cognitive function and then treated with EPO. The mice that received EPO demonstrated an improvement of cognitive function – thus, the treated mice had restoration of brain function. See Example 10 of specification starting at p. 114. Therefore, the meaning of the terms "restore" and "rejuvenate" as used in the specification do not require new cell growth.

Based on the above, claims 54-58 satisfy the enablement requirement. Applicants therefore respectfully request the withdrawal of the rejection of claims 54-58 under 35 U.S.C. § 112, first paragraph, for lack of enablement. Applicants further submit that, for the reasons set forth above, new claims 69-73 are also enabled.

V. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION, SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicants believe that the amendment of claims 54, 56 and 57 obviates this rejection for the reasons set forth below.

Specific mutein recombinant tissue protective cytokines that may be used in the claimed invention are described throughout the specification. For example, particular muteins within the scope of the claims as amended are provided at, *e.g.*, pp. 32-35 and 47-49 of the specification. Examples of specific EPO muteins that are reduced for erythropoietic activity compared to EPO are provided in the working examples of the specification (see Example 17). The tissue protective function of EPO muteins is also demonstrated by

working examples. See specification, Example 3 (S100E), Example 11 (S100E), Example 14 (R130E and R150E), Example 15 (S100E), Example 16 (S100E), and Example 18 (R103E, R150E, S100E).

Moreover, the instant specification discloses a correlation between the structure of the claimed muteins and their function. Specifically, the regions now defined in the claims affect erythropoietic activity of EPO. Mutations in one or more amino acids in this area yields a mutein having reduced erythropoietic activity compared to native recombinant human EPO. See specification at p. 4, l. 20 to p. 5, l. 2.

Therefore, the specification provides extensive description of the EPO muteins recited in the claims, methods for their use, and numerous working examples that demonstrate the correlation between the between structure of the EPO muteins and their function, such that a skilled artisan would recognize that Applicants were in possession of the claimed invention at the time of filing. Thus, the rejection under 35 U.S.C. § 112, first paragraph, for lack of written description should be withdrawn.

VI. THE REJECTIONS UNDER 35 U.S.C. § 102 SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 102(e), as allegedly being anticipated by International Application Publication No. WO 02/053580 to Brines *et al.*, filed on December 28, 2001. Applicants submit that International Application Publication No. WO 02/053580 does not anticipate the claims as amended because this reference generically discloses EPO muteins, but does not disclose the specific EPO muteins recited in the instant claims. This reference does not list the specific muteins recited in the instant claims, *i.e.*, EPO muteins with the particular structural and functional features required by the claims, such as mutations in 28 amino acids of the 165 amino acids of the mature protein. See specification at p. 39, ll. 9-12. Therefore, the broad genus of "mutein" disclosed in these references does not anticipate the specific, defined mutein species of the claims as amended. *Cf. Impax Laboratories, Inc. v. Aventis Pharmaceuticals Inc.*, 468 F.3d 1366 (Fed. Cir. 2006) (holding that a prior patent containing a formula including hundreds of compounds does not anticipate the specific compound of the claims, and the specific compound was not defined in the prior patent).

Accordingly, amended claims 54-58 are not anticipated by WO 02/053580 and Applicants respectfully request the withdrawal of this rejection.

Claims 54-58 are rejected under 35 U.S.C. § 102(b), as allegedly being anticipated by Campana *et al.*, 1998, *Int J Mol Med* 1(1):235041; abstract ("Campana"). Applicants believe that this rejection is in error. The EPO muteins of amended claims 54-58 require a substitution of an amino acid residue at one or more specific amino acid positions of the EPO protein. In contrast, Campana discloses a 17-mer EPO peptide, which could be generated by deletion of amino acids from the EPO sequence. Since the claims recite EPO proteins with alterations at specific amino acid positions, and the Campana peptide does not have any substituted amino acids, Campana does not anticipate the claims. Moreover, the claimed methods for protecting, maintaining or enhancing the viability of cells, tissues, or organs, treating or preventing diseases, and restoring or rejuvenating tissues or tissue function are not taught by Campana.

Therefore, Applicants respectfully request the withdrawal of the rejection of claims 54-68 for anticipation by Campana under 35 U.S.C. § 102(b).

VII. THE CLAIM REJECTIONS FOR DOUBLE PATENTING SHOULD BE CONTINUED TO BE HELD IN ABEYANCE

Claim 57 is rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-10 of U.S. Patent No. 6,531,121 B2.

Claims 54-58 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 35 and 37-38 of copending Application No. 10/188,905.

Claims 57-58 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 15 of copending Application No. 09/716,960.

Claims 54-56 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 2 and 5 of copending Application No. 10/351,640.

Claim 57 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of copending Application No. 10/185,841.

Claim 57 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 4 of copending Application No. 10/573,905.

In response Applicants request that the double patenting rejections continue to be held in abeyance until indication of allowable subject matter in the present application.

CONCLUSION

Entry of the foregoing amendments and remarks into the record of the above-identified application is respectfully requested. Applicants estimate that the remarks made herein place the pending claims in condition for allowance.

Respectfully submitted,

Date: June 30, 2008

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Enclosures

Appendix A: Replacement table for pages 69-71 of the specification

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
Heart	Ischemia	Coronary artery disease	Acute, chronic Stable, unstable
		Myocardial infarction	Dressler's syndrome
		Angina	
		Congenital heart disease	Valvular Cardiomyopathy
		Prinzmetal angina	
		Cardiac rupture	Aneurysmatic Septal perforation
		Angiitis	
	Arrhythmia	Tachy-, bradyarrhythmia *Supra*ventricular, ventricular Conduction abnormalities	Stable, unstable Hypersensitive carotid sinus node
	Congestive heart failure	Left, right, bi-ventricular, systolic, diastolic	Cardiomyopathies, such as idiopathic familial, infective, metabolic, storage disease, deficiencies, connective tissue disorder, infiltration and granulomas, neurovascular
		Myocarditis	Autoimmune, infective, idiopathic
		Cor pulmonale	
	Blunt and penetrating trauma		
	Toxins	Cocaine toxicity	
Vascular	Hypertension	Primary, secondary	
	Decompression sickness		
	Fibromuscular hyperplasia		
	Aneurysm	Dissecting, ruptured, enlarging	
Lungs	Obstructive	Asthma Chronic bronchitis, Emphysema and airway obstruction	
	Ischemic lung disease	Pulmonary embolism, Pulmonary thrombosis, Fat embolism	
	Environmental lung diseases		
	Ischemic lung disease	Pulmonary embolism Pulmonary thrombosis	
	Interstitial lung disease	Idiopathic pulmonary fibrosis	
	Congenital	Cystic fibrosis	
	Cor pulmonale		
	Trauma		

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
	Pneumonia and pneumonitides	Infectious, parasitic, toxic, traumatic, burn, aspiration	
	Sarcoidosis		
Pancreas	Endocrine	Diabetes mellitus, type I and II	Beta cell failure, dysfunction Diabetic neuropathy
		Other endocrine cell failure of the pancreas	
	Exocrine	Exocrine pancreas failure	pancreatitis
Bone	Osteopenia	Primary secondary	Hypogonadism immobilisation Postmenopausal Age-related Hyperparathyroidism Hyperthyroidism Calcium, magnesium, phosphorus and/or vitamin D deficiency
	Osteomyelitis		
	Avascular necrosis		
	Trauma		
	Paget's disease		
Skin	Alopecia	Areata Totalis	Primary Secondary Male pattern baldness
	Vitiligo	Localized generalized	Primary secondary
	Diabetic ulceration		
	Peripheral vascular disease		
	Burn injuries		
Autoimmune disorders	Lupus erythematodes, Sjiogren, Rheumatoid arthritis, Glomerulonephritis, Angiitis		
	Langerhan's histiocytosis		
Eye	Optic neuritis		
	Blunt and penetrating injuries, Infections, Sarcoid, Sickle C disease, Retinal detachment, Temporal arteritis		

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
	Retinal ischemia, Macular degeneration, Retinitis pigmentosa, Arteriosclerotic retinopathy, Hypertensive retinopathy, Retinal artery blockage, Retinal vein blockage, Hypotension, Diabetic retinopathy, and Macular edema		
Embryonic and fetal disorders	Asphyxia		
	Ischemia		
CNS	Chronic fatigue syndrome, acute and chronic hypoosmolar and hyperosmolar syndromes, AIDS Dementia, Electrocution		
	Encephalitis	Rabies, Herpes	
	Meningitis		
	Subdural hematoma		
	Nicotine addiction		
	Drug abuse and withdrawal	Cocaine, heroin, crack, marijuana, LSD, PCP, poly-drug abuse, ecstasy, opioids, sedative hypnotics, amphetamines, caffeine	
	Obsessive-compulsive disorders		
	Spinal stenosis, Transverse myelitis, ~~Guillian~~ Guillain Barre, Trauma, Nerve root compression, Tumoral compression, Heat stroke		
ENT	Tinnitus Meuniere's syndrome Hearing loss		
	Traumatic injury, barotraumas		
Kidney	Renal failure	Acute, chronic	Vascular/ischemic, interstitial disease, diabetic kidney disease, nephrotic syndromes, infections, injury, contrast-induced, chemotherapy-induced, CPB-induced, or preventive
	Henoch S. Purpura		
Striated muscle	Autoimmune disorders	Myasthenia gravis Dermatomyositis Polymyositis	

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
	Myopathies	Inherited metabolic, endocrine and toxic	
	Heat stroke		
	Crush injury		
	Rhabdomylosis		
	Mitochondrial disease		
	Infection	Necrotizing fasciitis	
Sexual dysfunction	Central and peripheral (e.g. erectile dysfunction)	Impotence secondary to medication, (diabetes)	
Liver	Hepatitis	Viral, bacterial, parasitic	
	Ischemic disease		
	Cirrhosis, fatty liver		
	Infiltrative/metabolic diseases		
Gastrointestinal	Ischemic bowel disease		
	Inflammatory bowel disease		
	Necrotizing enterocolitis		
Organ transplantation	Treatment of donor and recipient		
Reproductive tract	Infertility	Vascular Autoimmune Uterine abnormalities Implantation disorders	
Endocrine	Glandular hyper- and hypofunction		

EXPRESS MAIL NO.: EV 654 896 264 US
Box 1 of 2, Refs. A73-A81, B09-B27; Box 2 of 2. Refs. B28-B43, C123-C239

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Nielsen *et al.*

Confirmation No.: 5726

Serial No.: 10/612,665

Art Unit: 1649

Filed: July 1, 2003

Examiner: Dutt, Aditi

For: RECOMBINANT TISSUE PROTECTIVE CYTOKINES AND ENCODING NUCLEIC ACIDS THEREOF FOR THE PROTECTION, RESTORATION AND ENHANCEMENT OF RESPONSIVE CELLS, TISSUES AND ORGANS

Attorney Docket No: 10165-022-999

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.56 AND §1.97

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure imposed by 37 C.F.R. § 1.56 and §1.97 to inform the Patent and Trademark Office of all references coming to the attention of each individual associated with the filing or prosecution of the subject application, which are or may be material to the patentability of any claim of the application, Attorneys for Applicants hereby invite the Examiner's attention to references A36-A81, B09-B43, and C123-C239 listed on the attached revised form PTO 1449 entitled "List of References Cited by Applicant."

Copies of References A73-A81 and B09-B27 are submitted in Box 1 under Express Mail No. EV654896264US; copies of references B28-B43 and C123-C239 are submitted in Box 2 under Express Mail No. EV654896278US.

Copies of references A36-A72 are not submitted herewith because they are U.S. patents or U.S. patent application publications. Pursuant to 37 C.F.R. § 1.98 (a)(2)(ii), the requirement for providing a copy of each U.S. patent or U.S. patent application publication

listed in an Information Disclosure Statement in a patent application, regardless of the filing date of the application, is eliminated.

The U.S. patent application listed as reference A77 is a national stage application of the international patent application listed as reference B35. Therefore, a complete copy of reference B35 is not submitted herewith because it is identical and therefore cumulative to the international patent application publication included with A77. The U.S. patent application listed as reference A79 is a national stage application of the international patent application listed as reference B42. Therefore, a complete copy of reference B42 is not submitted herewith because it is identical and therefore cumulative to the international patent application publication included with A79.

Identification of the listed references is not meant to be construed as an admission of Applicants or Attorneys for Applicants that such references are available as "prior art" against the subject application.

Applicants respectfully request that the Examiner review the listed references and that the references be made of record in the file history of the application.

Pursuant to 37 C.F.R. § 1.97(c), since this Information Disclosure Statement is being filed after the mailing date of a first Office Action on the merits, but before the mailing date of a final action under 37 C.F.R.§ 1.311 or an action that otherwise closes prosecution in this application, it is estimated that a fee of **$180.00** as set forth in 37 C.F.R. § 1.17(p) is due for this submission. Please charge the required fee to Jones Day deposit account no. 50-3013. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

Date: June 30, 2008

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Enclosures

LIST OF REFERENCES CITED BY APPLICANT
(Use several sheets if necessary)

ATTY. DOCKET NO.	APPLICATION NO.
10165-022-999	10/612,665
APPLICANT	
Nielsen et al.	
FILING DATE	ART UNIT
July 1, 2003	1649

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	PAGES, COLUMNS, LINES, WHERE RELEVANT PASSAGES OR RELEVANT FIGURES APPEAR
	A36	4,992,419	2/12/91	Woog et al.	
	A37	5,824,672	10/20/98	Simpkins et al.	
	A38	6,242,570	6/05/01	Sytkowski	
	A39	6,340,742	1/22/02	Burg et al.	
	A40	6,521,245	2/18/03	Zaharia	
	A41	6,531,121	3/11/03	Brines et al.	
	A42	6,583,272	6/24/03	Bailon	
	A43	6,673,575	01/06/04	Franze	
	A44	7,214,532	5/08/07	Stern et al.	
	A45	7,309,687	12/18/07	Brines et al.	
	A46	7,345,019	03/18/08	Brines et al.	
	A47	2002/0031806	3/14/02	Lee	
	A48	2002/0052309	5/02/02	Anagnostou et al.	
	A49	2002/0061849	5/23/02	Nielsen et al.	
	A50	2002/0077294	6/20/02	Kay et al.	
	A51	2002/0081734	6/27/02	Choi et al.	
	A52	2002/0086816	7/04/02	Brines et al.	
	A53	2003/0077753	4/24/03	Tischer	
	A54	2003/0083251	5/01/03	Westfenfelder	
	A55	2003/0104988	6/5/03	Brines	
	A56	2003/0113871	6/19/03	Lee et al.	
	A57	2003/0120045	6/26/03	Bailon	
	A58	2003/0124115	7/3/03	Lee et al.	
	A59	2003/0134798	7/17/03	Brines et al.	
	A60	2003/0166566	9/04/03	Kinstler et al.	
	A61	2004/0009902	1/15/04	Boime	
	A62	2004/0018978	1/29/04	Campana et al.	
	A63	2004/0091961	5/13/04	Evans et al.	
	A64	2004/0096447	5/20/04	Yasuda et al.	

EXAMINER NYI-4081908v2	**DATE CONSIDERED**

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with **MPEP 609**; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

LIST OF REFERENCES CITED BY APPLICANT (Use several sheets if necessary)	ATTY. DOCKET NO. 10165-022-999	APPLICATION NO. 10/612,665
	APPLICANT Nielsen et al.	
	FILING DATE July 1, 2003	ART UNIT 1649

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	PAGES, COLUMNS, LINES, WHERE RELEVANT PASSAGES OR RELEVANT FIGURES APPEAR
	A65	2004/0209812	10/21/04	Farrell et al.	
	A66	2004/0214236	10/28/04	Brines et al.	
	A67	2005/0176627	08/11/05	Cerami et al.	
	A68	2006/0034799	2/16/06	Brines et al.	
	A69	2006/0216757	9/28/06	Brines et al.	
	A70	2007/0129293	6/07/07	Coleman et al.	
	A71	2007/0298031	12/27/07	Brines et al.	
	A72	2008/0045412	02/21/08	Brines et al.	
	A73	09/547,220	4/11/00	Brines et al.	
	A74	09/716,960	11/21/00	Brines et al.	
	A75	09/716,963	11/21/00	Brines et al.	
	A76	09/718,829	11/21/00	Brines et al.	
	A77	10/554,517	10/25/05	Brines et al.	
	A78	11/283,024	11/18/05	Cerami et al.	
	A79	11/631,458	1/03/07	Cerami et al.	
	A80	11/880,275	7/19/07	Brines et al.	
	A81	12/123,828	5/20/08	Brines et al.	

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL*		FOREIGN PATENT DOCUMENT COUNTRY CODE, NUMBER, KIND CODE (IF KNOWN)	DATE	NAME	PAGES, COLUMNS, LINES, WHERE RELEVANT PASSAGES OR RELEVANT FIGURES APPEAR	T
	B09	DE 198 57 609 (with translation)	6/15/00	Ehrenreich and Gleiter		
	B10	EP 0640619	8/16/94	Amgen Inc.		
	B11	EP 0668 351	8/23/95	Amgen, Inc.		
	B12	EP 1064951	6/28/00	F. Hoffmann-La Roche AG		
	B13	WO 85/02610	6/20/85	Kirin-Amgen, Inc.		
	B14	WO 86/03520	6/19/86	Genetics Institute, Inc.		
	B15	WO 91/05867	5/02/91	Amgen Inc.		

EXAMINER NY1-4081908v2	**DATE CONSIDERED**

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with **MPEP 609**; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL*		FOREIGN PATENT DOCUMENT COUNTRY CODE, NUMBER, KIND CODE (IF KNOWN)	DATE	NAME	PAGES, COLUMNS, LINES, WHERE RELEVANT PASSAGES OR RELEVANT FIGURES APPEAR	T
	B16	WO 92/08493	5/29/92	Brigham & Women's Hospital		
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	B20	WO 98/10650	3/19/98	East Carolina University		
	B21	WO 99/21966	5/06/99	Neurospheres Holdings Ltd.		
	B22	WO 00/24893	5/4/00	Amgen, Inc.		
	B23	WO 00/032772	6/8/00	Eli Lilly & Co.		
	B24	WO 00/061164	10/19/00	Kenneth S. Warren Laboratories		
	B25	WO 01/02017	1/11/01	F. Hoffmann-La Roche AG		
	B26	WO 01/81405	1/11/01	Amgen Inc.		
	B27	WO 01/87329	11/22/01	F. Hoffmann-La Roche AG		
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	B29	WO 02/14356	2/21/02	Althoff, Claudia		
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	B31	WO 03/029291	4/10/03	F. Hoffmann-La Roche AG		
	B32	WO 04/003176	1/8/04	The Kenneth S. Warren Institute, Inc. and H. Lundbeck A/S		
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EXAMINER NYI-4081908v2	**DATE CONSIDERED**

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with **MPEP 609**; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.